Developmental Variability and Heterochronic Evolution in Poeciliid Fishes (Cyprinodontiformes)

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The relationship between developmental variability and evolutionary diversity has become a major topic of concern and debate in evolutionary biology (Gould, 1977; Bonner, 1982; Goodwin et al., 1983; Humphries, 1988; Thomson, 1988). Recent studies of organismic development, for example, have focused on the genetic and epigenetic bases of evolutionary novelties (Raff and Kaufman, 1983), on the ontogenetic bases of phenotypic plasticity (Smith-Gill, 1983; Meyer, 1987) and life-history evolution (Stearns, 1982), and on the role of developmental constraints in phenotypic evolution (Alberch, 1980, 1982).

Developmental variability is also an important issue to systematists who have attempted to relate variations in ontogeny to methods of assessing phylogenetic relationships and understanding character evolution. Such work is motivated by the hypothesis that morphological diversity among species can arise from heterochronic changes in the development of the components of morphological form, specifically the sizes and shapes of organisms and the structures of which they are composed (Alberch et al., 1979; Kluge and Strauss, 1985; Atchley, 1987). Because heterochrony is thought to be an important mechanism of evolutionary change in many organisms, it has led to exploratory and experimental studies of the developmental processes underlying morphological changes during evolution (Maderson, 1975; Alberch, 1980; Alberch and Alberch, 1981; Raff and Kaufman, 1983; Atchley, 1984, 1987; Hall, 1984; Creighton and Strauss, 1986; Slatkin, 1987). Most of the vast literature on heterochrony concerns the classical cases of paedomorphosis (the occurrence of ancestral larval characteristics in adults of extant species) and recapitulation (evolution via the addition of new terminal stages onto ancestral developmental sequences). However, it has increasingly been recognized that many of the species-specific differences that we observe among adult forms may be accounted for by continuous variation in developmental timing processes that are neither strictly paedomorphic nor recapitulatory (Alberch et al., 1979; Kluge and Strauss, 1985).

Furthermore, such developmental changes may retain a significant historical or phylogenetic component (Creighton and Strauss, 1986).

The poeciliid fishes are a diverse, taxonomically and ecologically important group of North, Middle, and South American freshwater fishes. They have been intensively studied over the past hundred years and much is known about their basic biology and systematics (Meffe and Snellson, 1989). Because many species are easily bred and raised they should be very useful in studies of heterochronic evolution. However, only the broad outlines of the development and growth of a few species have been documented (e.g., Grobstein, 1940; Mookerjee et al., 1940; Scrimshaw, 1944, 1945; Tavolga and Rugh, 1947; Tavolga, 1949; Rosen and Kallman, 1959; Weisel, 1967). Moreover, almost nothing is known about the functional and historical connections between interspecific differences in development and adult morphology in these or other fishes.

The purpose of this paper is to summarize some preliminary information about ontogenetic variation in several species of poeciliids, and to suggest a methodological context in which such variation can be used to assess phylogenetic relationships and to better understand the evolution of morphological differences among species. The patterns of developmental variability described here derive from an ongoing study of ontogenetic evolution in poeciliid fishes and of the phylogenetic information inherent in such patterns of variability. Specifically, the study has the following objectives:

1. To quantify and compare patterns of differential allometric growth among species. It is axiomatic that interspecific differences in adult body form must be a function of differences in allometries and of the ways in which such growth gradients shift during development, such as during larval transformation and at the onset of sexual dimorphism.

2. To quantify and compare developmental relationships among osseous skeletal elements in terms of times of appearance (ossification onset) and relative growth rates. The detailed study of individual skeletal elements is necessary not only to understand how body form at any particular ontogenetic stage is a function of interactions among bones and bone complexes, but also to characterize interspecific differences in adult osteology in terms of historical patterns of developmental character change.

3. To determine the extent to which developmental traits can provide more highly resolved assessments of relationships among species. A preliminary step is to determine whether cladograms derived from ontogenetic data are at least congruent with corresponding cladograms based upon static adult morphology. Such crude congruence tests provide measures of the strength and consistency of the phylogenetic component of developmental variation.

4. To understand the ways in which these developmental patterns constrain or facilitate morphological change over evolutionary time and the resulting diversity among adult forms, within the context of hypothesized phylogenetic relationships.

There are many potentially important aspects of developmental variability that might be quantified and compared. However, initial concentration on the morphogenetic characteristics of bony elements and overall body form (a product of
interactions among skeletal elements and soft tissues as well as the sizes and shapes of individual bones) is warranted for two reasons: because of the overwhelming importance of skeletal traits in conventional systematic studies of these and other fishes, and because of the potential importance of being able to describe morphological differences among adults in terms of heterochronic ontogenetic changes (i.e., evolutionary changes in the timing of developmental events and rates of development). Indeed, it has often been claimed that variation in skeletal growth has been one of the most important agents of evolutionary change in cranial taxa (Hanken and Hall, 1983; Løvtrup, 1988).

Significance of the Poeciliids

The "poeciliid" fishes (family Poeciliidae of Rosen and Bailey [1963] and Rosen [1964, 1979]; subfamily Poeciliinae of Parenti [1981]) comprise one of the dominant aquatic groups of Middle America and the West Indies (Meffe and Snelson, 1989). They also extend into south-central and southeastern United States and into South America to the Rio de la Plata drainage. The poeciliids are undoubtedly monophyletic (Parenti, 1981; Parenti and Rauchenberger, 1989). Rauchenberger (1989) lists 194 currently recognized species in 22 genera.

Many poeciliids are easily kept and propagated in captivity, and have become exceedingly valuable research animals. More technical information has been accumulated on them than on any other family of fishes (with the possible exception of salmonids), and they have provided vast amounts of data on subjects ranging from genetics, evolution, and biogeography to cancer research. Their considerable structural diversity has made them important in evolutionary studies of adaptive specialization.

The poeciliids are particularly valuable subjects for studies of developmental variability and heterochronic evolution, for several reasons.

1. The group is demonstrably monophyletic (distinguished in particular by the structure of the male gonopodium) yet phenotypically diverse, especially in skeletal morphology. Its sister groups are uncertain, however, as are many generic assignments and relationships within the group (Rosen and Bailey, 1963; Rosen, 1964; Parenti, 1981; Parenti and Rauchenberger, 1989), but reasonably well corroborated phylogenies have been developed for several lineages based on morphological (primarily osteological) and genetic information.

2. Natural populations of many species are accessible in a relatively undisturbed state.

3. Most species are amenable to laboratory culture and experimental manipulation.

4. A few species have been studied sufficiently for there to be available basic data on morphology, genetics, and life-history characteristics.

5. Poeciliids for the most part are ovoviviparous, display continuous and semideterminant growth with rapid growth rates, and are easy to breed and maintain in aquaria. Broods are produced approximately once per month in most species, with from 10 to more than 90 fry per brood. Growth rates may be extrinsically controlled, primarily by temperature and secondarily by food availability.
MATERIALS AND METHODS

Study Organisms

Ossification patterns have been studied in five species of poeciliids, including four species of the subfamily Poeciliinae of Rosen and Bailey (1963): *Xiphophorus helleri* (Green Swordtail), *Xiphophorus maculatus* (Southern Platyfish), *Poecilia reticulata* (Guppy), and *Poecilia latipinna* (Sailfin Molly); and one species of the subfamily Heterandriini: *Poeciliopsis occidentalis* (Gila Topminnow). These species were selected to form a nested phylogenetic design representing three lineages (genera) of varying evolutionary distinctiveness, two of which were represented by two species each for within-lineage comparisons. Additional but incomplete ontogenetic data were also examined for *Gambusia affinis* (Mosquitofish) and *Brachyrhaphis epicopi* (Bishop).

Parental stocks for the present study were obtained from commercial sources (*X. helleri*, *X. maculatus*, and *P. latipinna*) or from natural populations (*P. reticulata* from Trinidad, *P. occidentalis* and *Gambusia affinis* from Sonoran Arizona, and *Brachyrhaphis epicopi* from Nicaragua). Fish stocks were maintained in aerated glass aquaria with a standardized lighting (12h on:12h off) and temperature (25–26 °C) regime and were fed daily to satiation with a standard diet of dry prepared food (Tetramin®) augmented by newly hatched brine shrimp. For developmental studies, batches of fry from a particular parental mating were raised in isolation, divided into two groups randomly assigned to different aquaria to control for ‘tank’ effects on growth. Multiple broods were raised for each parental pair. Individuals from sibling cohorts were sequentially sampled over time and preserved at regular intervals to obtain sufficiently resolved size and age series. The oldest fry were raised for approximately 3 months, to a standard body length (SL) of 15–20 mm in the smaller species and 20–25 mm in the larger species. After approximately six months of breeding, female parents were sacrificed about 1 week prior to parturition and prenatal embryos were dissected from the ovaries. To increase sample sizes, additional embryos were recovered from females in various stages of gestation.

Fry and adults were sacrificed by overdose of tricaine methanesulfonate (MS-222) and were fixed and stored in 10% buffered formalin. All specimens of embryos, fry, and adults were photographed (with metric scale) in lateral and dorsal projection for morphometric study, using an Olympus SZH dissecting microscope fitted with a photographic tube and camera. Specimens for osteological study were subsequently cleared with hydrogen peroxide, trypsin, and potassium hydroxide, and counterstained using Alizarin Red S for bone and Alcian Blue for cartilage (*Dingerkus and Uhler, 1977; Balon and Flegler-Balon, 1985*). Cleared-and-stained specimens were then re-photographed for additional morphometric study.

Allometric Growth

Basic morphometric data were obtained by digitizing point coordinates of anatomical landmarks judged to be homologous from form to form, and then computing linear distances between selected pairs of landmarks (Fig. 1). Interpoint distances were selected to form triangles and trusses (*Strauss and Bookstein, 1982*), geometric arrangements that are highly sensitive to changes in size and shape among forms, with additional nonredundant distances added as required. It is often difficult to reliably locate such landmarks in larval fishes due to their relatively
undifferentiated morphological structures at small sizes (Strauss and Fuiman, 1985), but in these species homologous interpoint distances can be reliably measured on series of individuals ranging from adults down to larvae 6 mm or so in total length. Auxillary measurements of lengths and widths of individual bones or bone complexes were measured directly on cleared-and-stained specimens using the same Olympus SZH dissecting microscope, fitted with an ocular micrometer.

For multivariate analyses, all measurements were converted to natural logarithms. The logarithmic transformation has several beneficial effects on the data structure: for individual characters, the variances become independent of their mean values (and thus comparable to one another in terms of intrinsic variability) to the extent that the individual coefficients of variation are constant (Lewontin, 1966; Lande, 1977); among characters, exponential bivariate and multivariate allometric relationships become colinear, with slopes that characterize relative growth rates with respect to body size (Bookstein et al., 1985). A general size factor was estimated separately for each species as the first principal component (PC1) of the within-group covariance matrix.

Although poeciliids do not pass through a relatively discrete larval “metamor-
Figure 2.
Example of the bilinear model (solid line) used to fit larval-to-adult allometric trajectories of log-characters on general size. The inflection point (open circle) was allowed to vary within the range of the data to maximize the variance explained by the model, which was assessed for statistical significance in relation to the variance explained by the corresponding linear model (dashed line).

General Size (S)

phasis,” as do other fishes that reorganize their growth gradients at some point during ontogeny (Strauss and Fuiman, 1985), allometric relationships were observed to change dramatically in these species during early growth. For this study allometric shifts were measured in the context of a bilinear spline model (Fig. 2), fitted to the data by functional regression based on orthogonal residuals. Using Powell’s direction-set optimization method (Press et al., 1986), the inflection point was allowed to vary within the range of data to maximize the variance explained by the model. The statistical significance of the bilinear model was assessed in relation to the standard linear allometric model. Because the bilinear model has two more parameters than the linear model, it will always provide at least as good a fit (i.e., explain at least as much variance); therefore its significance was estimated using an improvement-of-fit F-statistic (Neter et al., 1985:94–96):

$$F = \frac{\text{SSE}_R - \text{SSE}_F}{df_R - df_F} + \frac{\text{SSE}_F}{df_F}$$

where $\text{SSE}_F$ and $\text{SSE}_R$ are the error sums-of-squares for the full (bilinear) and reduced (linear) model, respectively, and $df_F$ and $df_R$ are the corresponding degrees of freedom. This statistic follows an $F$ distribution with $df_R - df_F$ and $df_F$ degrees of freedom and measures the relative improvement of fit in relation to the number of additional parameters.

When the bilinear model was significant for a character (the usual case in this study), the slopes of the two segments were used as estimates of its “larval” and
“adult” allometric coefficients. When the model was not significant the slope of the linear model, equivalent to the PC1 coefficient for that character, was used for both stages. All allometric coefficients were rescaled within each combination of stage and species to a mean of 1.0 (isometry). Such multivariate allometries are estimates of the relative rates of change (i.e., growth rates) of individual characters with general size, a robust measure of biological age (Strauss, 1987). Thus, an allometric coefficient of exactly 1.0 would then indicate isometry (unit change in the character for a unit in body size). Coefficients significantly greater than unity describe positive allometry (a relative increase in the character with increase in size), while those significantly less than unity indicate negative allometry (relative decrease in the character with size). Characters were judged to be isometric (the null hypothesis) when they were neither positively nor negatively allometric, based on standard errors of the slopes. If an organism were growing isometrically (a rare condition), it would not change its body shape during growth. Allometrically growing organisms alter their body proportions as they become larger.

Differential Ossification

The 17 bones and bone complexes utilized and the rationales for selecting them were described by Strauss (1990a). Most of the bones studied were cranial or visceral, but a few postcranial axial and appendicular bones were included.

Ossification in poecilids begins in embryos of about 4 mm length and continues through late juvenile stages. Ossification sequences were determined by examining large numbers of fry sequentially preserved at various stages of development, from late embryo to mid-juvenile. For each individual, any bony element that had appeared or been modified with respect to all smaller specimens was recorded as first occurring at the size of that individual. Degree of development of many bones, particularly dermal and vertebral structures, can be judged from cleared-and-stained specimens, but the developmental status of many of the inner cranial bones can be judged only by sectioning the fry and examining cellular structure under high magnification. Sequences in which selected bone ossifications occur during development (Weisel, 1967; Moser and Ahlstrom, 1970; Langille and Hall, 1987) were then mapped as a function of body size to produce ossification profiles that are directly comparable among species.

Because ossification takes place over a significant duration of total lifespan and must be studied using cross-sectional rather than longitudinal data, relatively large numbers of individuals must be observed in order to specify the time of ossification of any particular structure with reasonable accuracy. The sample sizes included in this study were sufficiently large to localize ossification times but not to statistically evaluate variability in timing. In addition, ossified tissues cannot always be recognized from stained preparations due to the vagaries of clearing-and-staining procedures. For these reasons minimum rather than mean body sizes were recorded for each ossification event and estimated ossification times are probably slight overestimates of the actual occurrences.

Cladograms and Character Mappings

Phylogenetic relationships based on continuous ossification timing data were assessed in two ways: with the parsimony (Wagner tree) procedure of Farris (1970),
using Farris's program HENNIG86, and with the restricted maximum-likelihood method of Felsenstein (1981). Felsenstein's algorithm is available as program CONTML in the PHYLIP (Phylogeny Inference Package) software, version 3.1. The parsimony and maximum-likelihood procedures differ significantly in their underlying assumptions. Basically, the parsimony method is designed to find the shortest dichotomous network connecting the taxa of interest, under the assumption that evolution has taken the shortest possible path with the minimum number of changes across all characters, while the maximum-likelihood procedure will find a unique connecting network based on the assumptions that evolution proceeds by "random walk" with a constant level of character variance through time. In both cases the resulting network must be rooted secondarily, usually by identifying the position at which an outgroup connects to the network. While both sets of assumptions are probably overly restrictive and unrealistic (Strauss, 1990b), parsimony methods seem to have a firmer philosophical foundation (Farris, 1983; Sober, 1984; Swofford and Berlocher, 1987) and have generally been preferred by systematists. Both methods were used in this study to determine the effects of the differing sets of assumptions on resultant tree topologies.

The optimal mapping of continuous timing states on resulting phylogenies was performed using my program CONTMAP, which finds the sets of nodal character states that minimize the squared branch lengths of the tree (Strauss, 1990b). All analyses were executed on an IBM PC-AT microcomputer.

RESULTS

Patterns of Allometric Growth

For all species the size range among specimens was so large (often 10:1 or more) that the general-size axes, estimated as within-group PC1 vectors, consistently accounted for 98% or more of total morphometric variation. Such a result would generally provide a rationale for using the rescaled coefficients as multivariate allometric coefficients (Jolicoeur, 1963; Bookstein et al., 1985). However, patterns of allometric growth were observed to be significantly non-loglinear during early stages, both for general external body measurements and for measurements of individual bones or bone complexes (e.g., Fig. 3). Thus the bilinear allometric model was used to attain estimates of "larval" and "adult" allometries and to estimate the time of maximal transition between stages. It should be noted that, although this bilinear model fits the data quite well, it represents a convenient approximation to an ostensibly curvilinear transition from larval to juvenile growth.

In general, allometric growth gradients tend to be much more extreme in larvae than in adults and to vary among species, both in the actual stage-specific allometries and in the kind of transition between stages (Fig. 4). That is, growth rates tend to be highly allometric (negatively or positively) during early growth and then to stabilize to more isometric patterns of growth in juveniles and adults. Extreme allometries in larvae indicate that their external body forms change relatively rapidly with increases in size. In both larvae and adults, however, variation among the allometric growth rates of different characters is fairly continuous.

Figure 4 portrays interspecific allometric gradient changes for a representative set of 10 mensural characters. Although there are some differences in relative character allometries among species, particularly among adults, the amount of congruence is
Figure 3.
An example of allometric changes in the size (width) of a single cartilaginous structure (Meckel's cartilage) and its bony replacement (the dentary bone), as a function of degree of ossification, in five poeciliid species: 1, *Xiphophorus helleri*; 2, *X. maculatus*; 3, *Poecilia reticulata*; 4, *P. latipinna*; 5, *Poeciliopsis occidentalis*. (a) Allometric relationships before and after externally complete ossification in *P. reticulata*, showing scatter among individuals and the best-fitting bilinear functional regression. Each point represents the width of the dentary structure as a function of general size for one individual, with the developmental status of the
Figure 4.
Shifts in allometries between larvae and adults for ten representative characters across five species of poeciliids. Allometries for larvae and adults are the segment slopes of the best-fitting bilinear functional regressions on general size. Values at bottom are sample sizes. Characters and corresponding landmark endpoints (Fig. 1) are: 1, pre-dorsal length (3-4); 2, body depth (11-16); 3, post-orbital head length (5-6); 4, snout length (2-4); 5, head depth (3-8); 6, caudal peduncle depth (13-17); 7, dorsal-fin base length (11-12); 8, head length (2-6); 9, head width (6L-6R); 10, orbit diameter (4-5).

Table 1. Rank correlations (Kendall’s \( \tau \)) among species between larval allometries (below diagonal) and adult allometries (above diagonal), and within species between corresponding larval and adult allometries (on diagonal).

<table>
<thead>
<tr>
<th>Species</th>
<th>X. helleri</th>
<th>X. maculatus</th>
<th>P. reticulata</th>
<th>P. latipinna</th>
<th>P. occidentalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>X. helleri</td>
<td>-0.02</td>
<td>0.29</td>
<td>0.22</td>
<td>0.34</td>
<td>0.31</td>
</tr>
<tr>
<td>X. maculatus</td>
<td>0.92</td>
<td>0.28</td>
<td>0.47</td>
<td>0.51</td>
<td>0.19</td>
</tr>
<tr>
<td>P. reticulata</td>
<td>0.74</td>
<td>0.70</td>
<td>0.37</td>
<td>0.72</td>
<td>0.51</td>
</tr>
<tr>
<td>P. latipinna</td>
<td>0.69</td>
<td>0.78</td>
<td>0.83</td>
<td>0.22</td>
<td>0.42</td>
</tr>
<tr>
<td>P. occidentalis</td>
<td>0.55</td>
<td>0.59</td>
<td>0.68</td>
<td>0.63</td>
<td>-0.12</td>
</tr>
</tbody>
</table>

The allometric relationships are indicated by the slopes of the line segments, fitted by a bilinear model for cartilaginous and partially ossified individuals before inflection, and ossified individuals after inflection. (b) The corresponding relationship in all five species, including that of Figure 3a (species no. 3) for reference. Units of general size are arbitrary but directly comparable across species.
moderately high. Interspecific rank correlations (Kendall’s τ) among larval allometric patterns (across all external mensural characters; Table 1) range from 0.55 between *Xiphophorus helleri* and *Poeciliopsis occidentalis* to 0.92 between *X. helleri* and *X. maculatus*. Corresponding correlations among adult allometries range from 0.19 between *X. maculatus* and *P. occidentalis* to 0.72 between *Poecilia reticulata* and *P. latipinna*. Thus larval allometries tend to be more highly correlated among species (and thus more evolutionarily conservative) than are adult allometries.

Perhaps the most significant observation about intraspecific allometric variation is that larval and adult allometries seem to be decoupled, in the sense of varying almost independently among characters. Rank correlations between larval and adult allometric coefficients range from -0.12 in *P. latipinna* to 0.37 in *P. reticulata*, with most correlations being statistically insignificant. Thus, the relative growth rates of different characters in adults cannot necessarily be "predicted" from the corresponding growth rates of larval characters, suggesting that adult body forms are not acquired simply by extrapolating larval growth gradients. Rather, larval growth gradients seem to be reorganized in the process of establishing the gradients that eventually characterize the growth of juveniles and adults, in a manner similar to (if less extreme than) larval metamorphoses observed in many marine fishes (Strauss and Fulman, 1985) and other vertebrates (Harris, 1989). Adult form is as much a product of the pattern of change from larva to juvenile as of the growth rates of juveniles and adults.

Patterns of Differential Ossification

Ossification profiles for these species were described previously (Strauss, 1990a), but are summarized here. Observed developmental patterns were generally consistent with previous developmental studies of poeciliids (e.g., Tavolga and Rugh, 1947; Tavolga 1949; Weisel, 1967). Ossification could first be detected by gross histology at about 4 mm length and continued through the largest juveniles studied.

The ossification profiles of the five species, graphically portrayed in Figure 5, are highly concordant among species. For example, rank correlations of sequence (Table 2) ranged from a low of 0.93 between *Poecilia reticulata* and *Xiphophorus helleri* to a high of 1.00 between *X. helleri* and *X. maculatus*, while parametric correlations based on actual timing values varied from 0.93 between *Poeciliopsis occidentalis* and *X. helleri* to 1.00 between *P. latipinna* and *P. reticulata*. The latter, almost perfect correlation is particularly interesting in light of the approximately 3:1 difference in body length between adults of the two species.

Despite the high timing correlations, however, numerous sequence changes among events are evident in the profiles, particularly in intergeneric comparisons. Because no confidence intervals can be associated with these data, such reversals and rearrangements of ossification sequences should not be over-interpreted, especially among closely associated events. Nonetheless, the relatively large number of sequences that are conserved in congeners, but rearranged in other taxa, suggests that similarity in the timing of ossification has a significant historical component, assuming that the classification is a reflection of genealogical relatedness.

It is expected that such interspecific variability among ossification events might be responsible for morphological differences in skeletal elements among adults. Analyses of correlations between the relative timing of ossifications and the sizes of
Figure 5.
Ossification profiles for a selection of bones or bone complexes were observed in five poeciliid species. Each point represents the minimum size at which the indicated bone was observed to completely ossify. Lines connect corresponding bone-ossification events in different species. Numbers represent coded character state values.

Table 2. Rank correlations (Kendall’s τ, above diagonal) and parametric correlations (Pearson’s r, below diagonal) of ossification profiles among species.

<table>
<thead>
<tr>
<th>Species</th>
<th>X. helleri</th>
<th>X. maculatus</th>
<th>P. reticulata</th>
<th>P. latipinna</th>
<th>P. occidentalis</th>
</tr>
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<tbody>
<tr>
<td>X. helleri</td>
<td>—</td>
<td>1.00</td>
<td>0.93</td>
<td>0.93</td>
<td>0.95</td>
</tr>
<tr>
<td>X. maculatus</td>
<td>0.99</td>
<td>—</td>
<td>0.93</td>
<td>0.93</td>
<td>0.95</td>
</tr>
<tr>
<td>P. reticulata</td>
<td>0.98</td>
<td>0.97</td>
<td>—</td>
<td>0.99</td>
<td>0.95</td>
</tr>
<tr>
<td>P. latipinna</td>
<td>0.98</td>
<td>0.97</td>
<td>1.00</td>
<td>—</td>
<td>0.93</td>
</tr>
<tr>
<td>P. occidentalis</td>
<td>0.93</td>
<td>0.94</td>
<td>0.96</td>
<td>0.94</td>
<td>—</td>
</tr>
</tbody>
</table>

the resulting bony structures suggest that the correspondence between timing and form may be fairly predictable. Figure 6 illustrates four such correlations, including two of the highest observed (for the frontal and nasal bones) and two of the lowest (posttemporal and mesethmoid). Approximately half of the interspecific correlations thus far examined are statistically significant, and all are greater than $r = 0.5$. The general pattern observed is that, the relatively later an ossification event occurs, the absolutely larger the bone becomes at a particular body size. The relative size of the
bone in a particular adult is then a function of the size of that individual. Because correlations between timing and bone size would be weakened by differences in localized growth rates among species (e.g., differences in growth rates of single bones or bone complexes), the strengths of the relationships of Figures 3 and 6 suggest that growth rates of individual bones are instead relatively conservative among species.

If the growth rates of bony structures before and after ossification are evolutionarily conservative, this in turn implies that it should be possible to predict the morphological effects in adults of evolutionary (heterochronic) changes in ossification times during development. For example, if a mutation occurred in *Gambusia affinis* that delayed the ossification of its frontal bone (species no. 3 in Fig. 6a) from a general size of 0.9 to 1.0, we could predict that the resulting size of the frontal bone at any particular adult body size would resemble that of *Poecilia reticulata* and *Brachyrhaphis episcopi* (species no. 2 and no. 7, respectively). Assuming that the ossification times of other bones in the same cranial region would adjust accordingly and that we could predict their adult sizes in the same way, we should then be able to specify in detail the structural changes that would result from this evolutionary change in the timings of ossification.

**Cladograms and Character Evolution**

Indirect tests of phylogenetic content of these developmental-timing characteristics were performed by constructing networks of relationship among the five
Figure 7.
(a) Conventional representation of phylogenetic relationships among the five poeciliid species, based primarily on Rosen and Bailey (1963). (b) Maximum-likelihood (ML) tree based on the complete set of quantitative ossification timing sequences of bony structures, including those of Figure 5. (c) ML tree based only on larval allometric coefficients. (d) ML tree based only on adult allometric coefficients. All ML networks are arbitrarily rooted to be consistent in topology with the conventional tree.

species utilizing the larval and adult allometries and the ossification-timing data (Fig. 7). Because these characters are treated here as continuous, they are intrinsically considered to be ordered variables. Resulting parsimony and maximum-likelihood trees, though based on different sets of evolutionary and statistical assumptions, were identical in topology for each data set.

The maximum-likelihood resolutions are depicted in Figures 7b–d, rooted between *Poeciliopsis occidentalis* and the remaining species to be comparable with the conventional hypothesis of relationships (Fig. 7a, based on Rosen and Bailey [1963], Rosen [1979, 1964], and Parenti and Rauchenberger [1989]). The parsimony resolutions differ only slightly in a few branch lengths, and are otherwise identical. Although the conventional hypothesis is not phylogenetic *sensu strictu* because a comprehensive cladistic study has not yet been carried out at the suprageneric level, the genera *Xiphophorus* and *Poecilia* have long been considered to be members of a distinctive higher-level group (the tribe Poeciliini) different from that of the genus *Poeciliopsis* (tribe Heterandriini), and thus the relationships of Figure 7a at least have a firm morphological basis. Although the use of additional outgroups would permit
an independent unambiguous rooting of the networks of Figures 7b–d, data on related species (*Gambusia affinis* and *Brachyrhaphis episcopi*) are currently incomplete; thus the networks were tentatively rooted to be consistent with the conventional hypothesis.

Of the three sets of data used to construct trees, the ossification-timing data and larval allometries both produced trees identical in topology with the conventional hypothesis. In contrast, the tree based only on adult allometries is incongruent both with the conventional hypothesis and with the trees estimated from the other ontogenetic data. Although these results are based upon only five species from three relatively distinctive poeciliid lineages, they strongly suggest that quantitative patterns of early growth and development are sufficiently conservative to preserve information about phylogenetic relationships. In contrast, the allometric gradients characterizing juvenile and adult growth seem to be homoplastic, leading to convergence in overall form among species, and are not by themselves sufficient to establish genealogical relationships (assuming the historical relationships in Fig. 7a to be correct).

To examine patterns of developmental character evolution, the ossification timing states and larval and adult allometries were mapped as continuous characters onto the tree of Figure 7a. The results are summarized in discrete fashion (Fig. 8) by noting whether a timing event or rate significantly "accelerates" or "decelerates" along branches, assessed with respect to observed variation within genera.

For the ossification timing data, an "acceleration" was interpreted to be an evolutionary change in calcification of a bone to a time relatively earlier in development (with respect to body size) than that of the hypothetical ancestral condition (the common ancestor of *Poeciliopsis occidentalis* and the remaining species; Fig. 7a), while a "retardation" indicates calcification later in development. The results from the mapping of continuous states (Fig. 8a) are identical to results previously obtained by "gap-coding" the states and mapping them as discrete characters (Strauss, 1990a). The character mappings suggest an approximately equal number of acceleration versus retardation events, and nearly equal numbers of timing changes from the outgroup to *Poecilia* and to *Xiphophorus*, with about a third of the total changes being possessed by their putative common ancestor.

For the allometric data, an acceleration was interpreted to be a shift in the relative growth rate of a character away from isometry, i.e., increasingly negatively or positively allometric with respect to the hypothetical ancestral condition. A shift away from isometry results in an increase in the rate of shape-change during growth. Conversely, a retardation was interpreted to represent a shift toward isometry. The resulting allometric character mappings (Fig. 8b) also suggest approximately equal numbers of acceleration versus retardation events, but with a larger proportion of larval changes occurring near the base of the tree and a larger proportion of adult changes occurring terminally.

**DISCUSSION**

This study has shown both allometric patterns and ossification profiles to be highly correlated among these poeciliid species, and thus evolutionarily conservative, both in sequence and in relative magnitude. Larval developmental patterns have long been hypothesized to be qualitatively conservative, and even these quantitative results are not surprising considering the complexity of the inductive
a Ossification timing

Poeciliopsis occidentalis

Xiphophorus helleri
Xiphophorus maculatus

Poecilia reticulata
Poecilia latipinna

○ Acceleration
● Retardation

b Larval and adult allometries

Poeciliopsis occidentalis

Xiphophorus helleri
Xiphophorus maculatus

Poecilia reticulata
Poecilia latipinna

Figure 8.

Parsimonious mapping of ossification and allometric changes onto the phylogenetic tree topology of Figure 7a, collapsed to the level of genus. Open circles represent accelerations in development with respect to body size; closed circles represent retardations. (a) Ossification timing changes for bones or bone complexes, with branch lengths proportional to numbers of discrete state changes. (b) Allometric (relative growth rate) changes for morphometric characters. Numbers of allometries increasing (open circles) and decreasing (closed circles) at each step are indicated above symbols; total number of changes is summarized below symbol pairs.
and morphogenetic interactions that control the development of complex structures such as the vertebrate head (Grobstein, 1967; Hall, 1987). Conservative ossification sequences are also to be expected on functional grounds; the bones first formed are often those that serve early functional demands and that may be subjected to the greatest muscular stresses (Weisel, 1960; Shaffer, 1961).

However, current theories of heterochronic evolution make no necessary predictions about the relationship between larval and adult developmental patterns in continuously growing organisms. Thus, two findings from this study are particularly important: (1) that corresponding larval and adult growth patterns seem to be evolutionarily decoupled (i.e., to vary almost independently) from each other among species, and (2) that larval allometric patterns are much more highly correlated among species, and thus more highly conserved and predictable, than are the corresponding adult allometries. Independence of stage-specific growth patterns is common in many marine fishes and other organisms that undergo larval metamorphoses, but has not been previously documented in freshwater fishes. The decoupling of juvenile and adult growth from the ostensibly phylogenetically conservative larval conditions in poeciliids permits a significant degree of convergence in body form among adults, which is often related to habitat (Rosen and Bailey, 1963).

Despite the interspecific congruence of larval allometric patterns and ossification sequences, the quantitative and qualitative differences among them appear to bear phylogenetic information, allowing construction of evolutionary hypotheses based directly on "dynamic" ontogenetic criteria rather than on static adult morphologies. This study is preliminary in that only a small number of taxa have been utilized, but the fundamental agreement between the estimates of relationship based on larval allometries and timing sequences on the one hand, and those based on more conventional morphological criteria on the other, suggests that dynamic ontogenetic traits should be at least as important as static adult characters for purposes of phylogenetic inference.

The use of rates and timing characters for phylogenetic inference is problematic, however, for two reasons. The first involves the usual difficulties of dealing with continuous rather than discrete data, necessitating either the conversion of continuous values to discrete states, either arbitrarily or by invoking an objective gap-coding criterion (Archie, 1985; Goldman, 1988), or the use of tree-building algorithms that either utilize distance matrices rather than character-state matrices or that are dependent on particular models of evolutionary change (Felsenstein, 1982). The second problem is that all current tree-building procedures are based on assumptions of character independence (Donoghue, 1989). The assumption that rate and timing traits are uncorrelated, and thus evolutionarily independent, is untenable on functional grounds.

A much more direct and informative use for such ontogenetic data is the mapping of rates and events onto a phylogenetic tree derived independently from other criteria (Strauss, 1990b). To the extent that the cladogram reflects the actual relationships and the character-state changes have been optimally positioned on the tree, a phylogenetic mapping predicts the historical sequence in which the ossification times have been modified from ancestral states (Lauder, 1982; Ridley, 1983; Donoghue, 1989). The use of timing and allometry changes in this study results in a direct portrayal of heterochronic evolution (Fig. 8), in the sense that state changes
can be directly interpreted as accelerations and retardations with respect to putative ancestral conditions. It is noteworthy that the putative timing and allometry changes of Figure 8 include approximately equal numbers of acceleration and retardation events, justifying Fink's (1982) contention that it is organismic traits, rather than whole organisms, that may evolve by heterochronic change.

By itself, a mapping of timing and rate changes tells us little about resultant patterns of morphological diversification. Work is currently under way to examine patterns such as those of Figure 6 more carefully, in order to assess and quantify the differences in adult morphology that result from evolutionary alterations in ossification sequence and timing. Because changes in growth rates during development have the potential to modify form significantly, they are an important source of variation on which natural selection might act. By evaluating, in the context of phylogenetic relationships, the patterns of morphological change during ontogeny and the ways in which they depend on developmental events, we might discern the way selection and other evolutionary processes have altered growth patterns to affect the variation that we observe in adult morphology. A secondary advantage of evaluating the ontogeny of morphological traits in the light of genealogical history is that instances in which similarity in adult structure results from different underlying developmental trends (ontogenetic convergence) can be identified (Creighton and Strauss, 1986).

This paper has summarized some initial but general findings about evolutionary diversification in the developmental patterns of several poeciliid species. The overall objective of this and ongoing research is to examine the ontogenetic changes in relative rates and timing that have occurred within a diverse monophyletic group of fishes, in order to assess both the ontogenetic changes associated with their morphological diversification and the constraints on developmental variability. Such studies of developmental and morphological diversification, as opposed merely to studies of variation, would not be possible without well established hypotheses of phylogenetic relationship. I believe that future work in this area should address the following major questions:

1. Which aspects of ontogeny (rates, timings, allometries) change during development and vary among taxa? That is, which components of developmental morphology are apparently constrained (i.e., limited in the extent or direction of change) and which are ostensibly free to vary? What are the intraspecific covariance relationships among these developmental variables, and to what degree do phenotypic covariance sets (particularly integrated subsets sensu Olson and Miller, 1958; Gould, 1984) differ among closely related versus distantly related species (as determined, for example, by cladistic relationships)?

2. What are the minimal ontogenetic changes (i.e., magnitudes of interspecific differences in rates or timings) necessary to account for observed morphological differences among the adults of different species? Are magnitudes of difference a function of phyletic divergence, or can relatively small ontogenetic changes be associated with large morphological differences? Is ontogenetic change predominantly diversifying, or can convergent similarities in size and shape of adult structures arise from differing patterns of development?
3. What are the developmental relationships between intraspecific polymorphism (sexual dimorphism, feeding morphs, reproductive morphs, etc.) and interspecific diversification?

4. Is intraspecific developmental variation (relative or absolute growth rates, phenotypic covariances) related to genetic variability, as estimated, for example, by individual heterozygosity? That is, how are developmental patterns within outbred populations altered by inbreeding?

Conventional descriptions of heterochronic evolutionary changes have been based on additions, deletions, and substitutions of ontogenetic stages (the "semaphoronts" of Hennig [1966]) among taxa (O'Grady, 1985; André, 1988), but there are a number of distinct advantages to describing life cycles in terms of models of growth and differentiation (Alberch et al., 1979; Kluge and Strauss, 1985; Kluge, 1988). Such developmental models are capable of representing all forms of variation (e.g., terminal and nonterminal, continuous and discrete, simple and complex). The quantitative nature of the models leads implicitly to greater precision and resolution. In particular, arbitrary divisions of the ontogenetic continuum into "stages" can be avoided. In fact, the discovery of discontinuities or irregularities in growth trajectories, such as the allometric larval transitions described here, can draw attention to subtle caenogenetic changes that might otherwise be ignored. The study of mixed cases of heterochrony is easily accomplished with developmental models but is more difficult or impossible when using discrete characters and stage comparisons.

The study of developmental variability at this stage is necessarily phenomenological and comparative, emphasizing quantitative descriptions and correlations of ontogenetic and morphological variation within and among species, in the context of hypothesized phylogenetic relationships. In this sense "heterochrony" implies an explanation rather than mechanism, a parsimonious set of inferences with which to account for observed correlations and limits to variation. However, study of both the patterns of variation and diversification among species and the mechanisms that produce them will be vital to an ultimate understanding of the relationships between development and evolution in poeciliids and other organisms.

SUMMARY

The quantitative description of allometric growth gradients, sequences of bone ossifications, and other developmental events provides in principle a basis for assessing the ontogenetic patterns underlying differences in morphological structure. To the extent that such patterns are evolutionarily divergent rather than homoplasious, they may also provide a basis for phylogenetic inference and the understanding of historical patterns of developmental character change. Allometric gradients and ossification-timing sequences were examined in laboratory-raised samples of five species of poeciliid fishes: Xiphophorus helleri, Xiphophorus maculatus, Poecilia reticulata, Poecilia latipinna, and Poeciliopsis occidentalis. These species were selected to represent three lineages (genera) of varying evolutionary distinctiveness.

Patterns of allometric growth were found to be significantly non-loglinear during early stages, with growth gradients in larvae being much more allometric than in juveniles and adults. Furthermore, larval and adult allometries are evolutionarily "decoupled" in the sense of varying almost independently among characters. Larval
allometries are significantly correlated (character by character) among species, suggesting that they are evolutionarily conservative, while adult allometries tend to be uncorrelated among species, suggesting relatively more variability and convergence in adult body form.

Ossification profiles are also highly correlated among species, but numerous sequence differences are evident, particularly in intergeneric comparisons. Bone ossifications correspond in many cases to localized shifts in allometric growth gradients, suggesting that it may be possible to predict the morphological effects in adults of heterochronic changes in ossification times during development.

The larval allometries by themselves, when used to construct a phylogenetic tree, contain sufficient historical information to recover evolutionary relationships consistent with a conventional phylogenetic hypothesis based on adult skeletal traits. A tree based on larval ossification-timing data is consistent with that based on larval allometric gradients, and thus with the conventional hypothesis. However, a corresponding tree based only on adult allometries is incongruent with the larval and conventional trees, again suggesting much homoplasy among adult forms. Mappings of these dynamic ontogenetic characters suggest approximately equal numbers of heterochronic acceleration and retardation events, with a larger proportion of larval changes occurring near the base of the tree and a larger proportion of adult changes occurring terminally.

LITERATURE CITED


